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Research Note—

Incidence and Tracking of Clostridium perfringens Through an Integrated Broiler Chicken Operation

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SUMMARY. *Clostridium perfringens* has been shown to be widespread in the broiler chicken hatchery, grow-out, and processing operations. In a previous study, ribotypes of certain strains of *C. perfringens* isolated from processed chicken carcasses were shown to match ribotypes isolated from paper pad lining trays used to transport commercial chicks from the hatchery to the grow-out facility on the farm. These results suggest that *C. perfringens* contaminating the processed product could originate from facilities in the integrated poultry operation prior to grow out. In this study, samples were collected from the breeder farm, hatchery, previous grow-out flock, during grow out and after processing. In the first trial, *C. perfringens* was recovered from the breeder farms, the hatchery, previous grow-out flock, grow-out flock at 3 weeks of age, grow-out flock at 5 weeks of age, from processed carcasses, and from the breeder farm after processing in 4%, 30%, 4%, 0%, 2% and 16%, and 4% of the samples, respectively. In the second trial, the incidence of *C. perfringens* in samples collected from breeder farms, the hatchery, previous grow-out flock, grow-out flock at 3 weeks of age, grow-out flock at 5 weeks of age, and from processed carcasses was 38%, 30%, 32%, 8%, 4%, and 8%, respectively. The genetic relatedness of the isolated strains as determined by ribotyping suggests that *C. perfringens* may be transmitted between facilities within the integrated broiler chicken operation.

RESUMEN. *Nota de Investigación*—Incidencia y seguimiento del Clostridium perfringens en una empresa integrada de pollos de engorde.

Se ha demostrado que el *Clostridium perfringens* esta ampliamente distribuido en las plantas de incubación, granjas de cría y plantas de procesamiento. En un estudio previo se demostró que los ribotipos de ciertas especies de *C. perfringens*, aislados a partir de muestras obtenidas de las canales de pollos a nivel de las plantas de procesamiento, eran iguales a los ribotipos de aislamientos de la bacteria obtenidos a partir de muestras del papel utilizado para cubrir el fondo de las bandejas usadas para el transporte de pollitos de un día de edad desde la planta de incubación hasta las granjas de cría. Estos resultados sugieren que la contaminación de productos procesados con aislados de *C. perfringens* puede originarse en etapas previas al periodo de cría en las integraciones avícolas comerciales. Para la realización de este estudio se tomaron muestras a partir de la granja de reproductoras, la planta de incubación, durante el periodo de cría de la parvada previa, durante el periodo de cría de la parvada experimental y al momento del procesamiento. En un primer experimento se aisló el *C. perfringens* a partir de muestras obtenidas en la granja de reproductoras, la planta de incubación, en la parvada anterior, la parvada experimental (a las 3 y 5 semanas de edad), a partir de las canales al momento del procesamiento y en la granja de reproductoras luego del procesamiento en un 4%, 30%, 4%, 0%, 2%, 16%, y 4% de las muestras tomadas, respectivamente. En un segundo experimento, la incidencia de *C. perfringens* en las muestras obtenidas a partir de la granja de reproductoras, la planta de incubación, en la parvada anterior, la parvada experimental (a las 3 y 5 semanas de edad) y a partir de las canales al momento del procesamiento fue de un 38%, 30%, 32%, 8%, 4% y 8%, respectivamente. Los niveles de relación genética en los aislados obtenidos, determinados mediante la técnica de ribotipificación, sugieren que el *C. perfringens* puede ser transmitido entre las diferentes instalaciones dentro de una integración avícola comercial.

Key words: *Clostridium perfringens*, broiler chickens, breeders, hatchery, grow out, processing plant

Abbreviations: BPW = buffered peptone water; CAMP = Christie, Atkins, Munch, and Peterson; TSC = tryptone-sulfite-cycloserine

Clostridium perfringens is recognized as an enteric bacterial pathogen in humans, poultry, other domestic animals, and wildlife worldwide (9) *Clostridium perfringens* has been isolated from processed broiler chickens and in the processing plant (8,4) and is reported to be a major cause of human food-borne disease outbreaks arising from the consumption of contaminated poultry and other meat products (1). Little is known about the distribution and sources of *C. perfringens* in poultry production facilities. Previously, we detected *C. perfringens* in a high percentage of paper pads lining the transport trays and collected after transport of chicks from the hatchery to the farm (6). Some of the isolates from paper pads were of the same ribotype as isolates from the associated processed broiler carcasses (unpubl. data). In another study, *C. perfringens* was isolated from shell fragments and chick fluff collected from the hatchers in commercial broiler chicken hatcheries (5). The objective of the present study was to determine the incidence of *C. perfringens* on the broiler breeder farm and associated hatchery, grow-out farm, and processed carcass samples. The relationship of the *C. perfringens* isolates from the geographically distinct facilities within an integrated operation were determined by ribotyping.

MATERIALS AND METHODS

Sample collection. *Sample locations.* In trial 1, samples were obtained from breeder A, the hatchery (containing breeder A eggs), broiler farm A (where breeder A chicks were placed), and the processing plant after processing the flock from broiler farm A. In trial 2, samples were obtained from the same breeder A as in trial 1 and a breeder B, the hatchery (containing breeder A and B eggs), broiler farm B (containing breeder A and B chicks), and the processing plant after processing the flock from broiler farm B.

Sample numbers. For each trial, the following numbers were obtained for each sample type from each location on the day of sampling: 50 fecal, 10 fluff, 10 egg shell, and 50 or 100 processed carcass rinses.

Fecal samples. Fecal droppings were collected from the broiler farms at week 5 (previous flock), and weeks 3 and 5 (current flock). Fecal droppings were collected from the breeder farms at the approximate time of egg setting for the current broiler flock. Fresh fecal droppings (single droppings of 5–10 g) were collected aseptically into disposable 50-ml centrifuge tubes (VWR brand, West Chester, PA) and transported to the laboratory on ice.

Hatchery samples. Hatchery samples (egg shells and fluff from the hatching cabinets) were collected on the day of hatch. Samples were aseptically placed into quart-size ziplock bags (Reynolds Metal Co., Richmond, VA) and transported to the laboratory on ice. Fluff samples (approximately 2 g each) were collected from the floor in front of and in the hatching cabinets. Egg shell fragments were collected from the hatching trays (top, middle, and bottom of the stack) with three to four shell fragments constituting a sample.

Carcass rinses. Carcass rinses were prepared using carcasses pulled from the line after they exited the chill tank. Each carcass was aseptically placed into a Cryovac bag (Cryovac, Inc., Simpsonville, SC); 100 ml of sterile distilled water was added to each bag (3); the bag was shaken for 60 sec; the carcass was replaced on the line; and the rinse water was poured into a sterile specimen cup (VWR Brand, West Chester, PA). The rinses were transported to the laboratory on ice.

Sample processing. *Preparation.* All fecal droppings were weighed and diluted 1:10 with buffered peptone water (BPW, Oxoid, Ltd., Basingstroke, Hampshire, England). Ten milliliters of BPW was added to all fluff samples, and the sample was thoroughly mixed by hand. Fifty milliliters of BPW was added to the crushed egg shell fragments (enough to fully cover the fragments in BPW). The appropriate amount of 10 × BPW was added to the carcass rinses to result in a 1 × BPW solution.

Microbiological methods. To tubes of modified iron milk medium (7), 4 ml of each sample was added and incubated at 37 C for 3 hr followed by incubation at 46 C for 15 hr. After incubation, a portion of the contents of tubes of iron milk medium demonstrating stormy fermentation or other gas formation were streak-plated onto tryptose-sulfite-cycloserine (TSC) agar with egg yolk (7). After anaerobic incubation provided by the AnaeroGen atmospheric generation system (Oxoid) at 37 C for 48 hr, typical colonies were picked and restreaked onto TSC agar and incubated as before. Where available, five typical colonies per sample were selected for testing. Isolated colonies were confirmed as *C. perfringens* as recommended by Harmon (7).

Ribotyping. For ribotyping, isolates were confirmed as *C. perfringens* using the reverse Christie, Atkins, Munch, and Peterson (CAMP) test (2). Confirmed *C. perfringens* strains were ribotyped using the Riboprinter Microbial Characterization System (Qualicon, Wil-

Table 1. Incidence and ribogroups of *Clostridium perfringens* isolated from samples collected in Trial 1.

Location	Week[A]	No. of positives[B]	Ribogroup			
			143-S8	313-S2	322-S1	Other
Breeder A	−3	2/50 (4)	—	—	—	1
Hatchery	0	24/80 (30)	1[C]	11	—	9
Broiler A	−4	2/50 (4)	—	1	—	—
Broiler A	3	0/50 (0)	—	—	—	—
Broiler A	5	1/50 (2)	—	1	—	—
Processing	6	16/100 (16)	—	1	—	—
Breeder A	7	4/100 (8)	—	1	2	—

[A]The week that hatchery samples were collected is considered week 0. The negative and positive numbers represent the number of weeks samples were collected at a given site before and after hatchery sampling, respectively.

[B]The numbers in parentheses represent the percentage of samples that were positive for *C. perfringens*.

[C]The number of samples from which this ribotype was isolated.

mington, DE) and kits containing *Eco*R1 enzyme for restriction endonuclease digestion and ligation.

RESULTS AND DISCUSSION

In the first trial, fecal samples from a single broiler breeder (parent) flock (breeder A) were positive for *Clostridium perfringens* in 2/50 (4%) of samples tested at the time that eggs from the breeder flock were placed in the hatching cabinet (Table 1). One ribotype was identified from these isolates. All isolates were not ribotyped because some became unculturable between the time of *C. perfringens* confirmation and ribotyping. Also for some samples positive for *C. perfringens* more than one ribotype was identified because multiple colonies originating from that sample were ribotyped. Composite samples from the hatching cabinet taken when the eggs were hatched (from breeder A eggs) were positive for *C. perfringens* in 24/80 (30%) samples with two ribotypes identified, none of which were found in the breeder samples. One of these two ribotypes (313-S2) was isolated from 11 hatchery samples. Fecal samples from the previous broiler flock housed in the sampled grow-out house were *C. perfringens* positive in 2/50 (4%) of the samples with one ribotype identified, 313-S2. At 3 and 5 weeks of grow out, fecal samples from the broiler flock A that followed were 0% and 2% *C. perfringens* positive with one ribotype identified (313-S2) that had also been detected in hatchery samples and in the previous broiler flock. Of the rinse samples of processed carcasses from broiler flock A, 16/100 (16%) were contaminated with *C. perfringens*. Of the two ribotypes identified from these carcasses, one (313-S2) had been previously isolated from the hatchery, in the previous broiler flock in the grow-out house and from broiler flock A 1 week before processing.

In the second trial, two breeder flocks (breeder A from the previous trial and a breeder B) were used to supply the birds for one broiler grow-out house (broiler flock B). The broiler house was different from the one used in the first trial and therefore referred to as broiler flock B. The two breeder flocks were positive for 32% and 44% of the fecal samples resulting in a mean contamination rate of 38% for fecal samples from the two breeder flocks used in this trial (Table 2). Two ribotypes were identified from breeder farm A and nine from breeder farm B. One of the ribotypes (313-S2) was common to the two farms and had also been isolated from breeder farm A, the hatchery, broiler farm A, and from a processed carcass in trial 1. Composite hatching cabinet samples were positive in 12/40 (30%) samples with six ribotypes identified, two of which, ribotypes 157-S7 and 169-S7, were previously isolated from breeder farm B. Of the fecal samples from the previous broiler flock, 35% were positive with six ribotypes isolated: 153-S2 and 153-S5 also isolated from breeder farm B, 168-S2 from breeder farm A, and 159-S7 from the hatchery. In broiler flock B, 8% and 4% of fecal samples were positive at weeks 3 and 5, respectively, and four ribotypes were identified: 143-S8 isolated from the previous broiler flock, 153-S5 isolated from the previous broiler flock and breeder farm B, 164-S7 isolated from breeder farm B, and 159-S7 isolated from the previous broiler flock and the hatchery. Ribotype 143-S8 had also been isolated from the hatchery in the previous trial. After processing, 4/50 (8%) of the

Table 2. Incidence and ribogroups of *Clostridium perfringens* isolated from samples collected in Trial 2.

			Ribogroup[D]												
Location	Week[A]	No. of positives[B]	143 S8	153 S2	153 S5	157 S7	159 S7	161 S6	164 S7	168 S2	169 S7	303 S6	310 S8	313 S2	313 S6	Other
Breeder A	−3	16/50 (32)	—	—	—	—	—	—	—	1[C]	—	—	—	1	—	—
Breeder B	−1	22/50 (44)	—	8	11	1	—	—	3	—	1	—	2	1	2	1
Hatchery	0	12/40 (30)	—	—	—	2	1	—	—	—	1	—	—	—	—	3
Broiler B	−3	17/49 (35)	2	1	8	—	3	—	—	1	—	—	—	—	—	1
Broiler B	3	4/50 (8)	—	—	2	—	2	—	—	—	—	—	—	—	—	—
Broiler B	5	2/50 (4)	1	—	—	—	—	—	1	—	—	—	—	—	—	—
Processing	6	4/50 (8)	—	1	—	—	1	2	—	—	—	2	—	2	—	1

[A]The week that hatchery samples were collected is considered week 0. The negative and positive numbers represent the number of weeks samples were collected at a given site before and after hatchery sampling, respectively.

[B]The numbers in parentheses represent the percentage of samples that were positive for *C. perfringens*.

[C]The number of samples from which this ribotype was isolated.

[D]Certain positive samples had more than one ribotype isolated from the sample.

carcasses were positive for *C. perfringens* with isolates from six different ribogroups: 153-S2 isolated from the previous broiler flock and the farm of breeder B, 159-S7 from broiler flock B at 3 weeks of age, the previous flock and the hatchery, and 313-S2 isolated from breeder farms A and B. Ribotypes 161-S6, 303-S6, and another ribotype had not previously been isolated but were isolated at processing.

These results indicate that *C. perfringens* can contaminate the breeder operation, hatchery, grow-out house, and processed carcasses of broiler chickens. None of the identified ribotypes could be traced directly from the breeder operation through the hatchery, the grow-out operation, and the processed carcass for a given trial. However, common ribotypes were isolated from the two breeder farms; the breeder farm and the hatchery; the two breeder farms and processed carcass; the breeder farms, broiler farms, and processed carcass; the breeder farm and the processed carcass; the hatchery and the broiler farm; and the hatchery, broiler farm, and processed carcass. One possible explanation for the failure to find a given ribotype in all facilities of the integrated operation is that not enough samples were evaluated. Another explanation is that populations of a given ribotype build up in the second facility slowly over a period of time before they are isolated. This pattern of contamination of *C. perfringens* was noted in a study of another broiler chicken integrator (unpubl. data). In that study, ribotypes of *C. perfringens* isolated from paper pads beneath chicks transported from the hatchery to the grow-out flock were often not isolated in the flock of chickens stocked with those chicks but were isolated in subsequent flocks reared in that grow-out house. The results in the current trial also support the results observed in the previous unpublished trial: some *C. perfringens* ribotypes are ubiquitous in the integrated operation, i.e., found in multiple facilities, and are persistent, i.e., isolated from samples over a period of time. Some *C. perfringens* ribotypes persisted in the current trial for 6 months or longer. The results of the current study indicate that at least some of the *C. perfringens* contamination found on processed broiler carcasses can originate in the breeder operation and be transmitted through the hatchery and grow-out operations.

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